

Steven M. Ruben
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Human Genome Sciences, Inc.
Lab Notebook # 443

Department Protein Expression
Subject IV
Name Marcus Bueglin
Address 9410 Kay Westlake Lab B1EC

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Computation Notebook

11 3/4" x 9 1/4", 4 x 4 Quad., 75 Sheets

43-648



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Human Genetic Sciences, Inc.
Lab Notebook #

Department Protein Expression
Subject IV
Name Martens Buerger
Address 9410 Regent St. LA 316

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Computation Notebook

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Ruben EXHIBIT 2055
Ruben v. Wiley et al.
Interference No. 105,077
RX 2055

12.1.95 Transfection of CHO-dhfr⁻ cells
with:

- 1.) BNP (N346 / BNP)
- 2.) TNF α (N346 / TNF α)
- 3.) TNF α (CHO / TNF α)

11.28.95 CHO dhfr⁻ cells confluent 25 plates
were diluted 1:10 and seeded into
the wells of a 6 well plate.

(- resuspend cells of T75 in 10ml
dissemination medium, seed 1ml
in each well of the 6 well plate.)

- incubate cells over night \rightarrow 90-95% confluent

11.29.95 - remove the medium of the 6 wells
- wash cells with PBS
- add 1 ml transfect. medium 1:
MEM 2+, 0% DMSO, 1% PS

prepare lipofectin-mix in 96 well plate:

A: 90 μ l transfect. medium 1 (6 wells)
10 μ l Lipofectin

B: 50 μ l transfect. medium 1
5 μ g Expression vector (N346 / BNP, N346 / TNF α ,
CHO-I / TNF α)
2 wells for each construct

1 μ g N18

- mix A + B and incubate 45' at RT

- add transfection mix drop wise to the cells in the 6 well
- incubate for 4-6 h at 37°C, 5% CO₂

- add 1ml transfect. med 2 to each well of
the 6 well plate: MEM 2+, 10% DMSO, 1% PS

- 12.1.95 seed the cells in selection medium

2 wells of a 6 well dish were transfected with one of the expression vectors

A) → seed cells of 1 well in selective medium containing 20 mM MTX.

B) → seed the cells of the second well transfected with the same expression vector in selective medium containing 100 mM MTX

Prepare selective medium:

- Have 1 ml of 5 mM MTX stock
 - dilute 1:50, 1:100 in MEM α - medium
 ⇒ [MTX] = 1 μ M

- Have 3 ml of G418 (100 mg/ml)

Selective Med. A: 20 mM MTX

Med. B: 100 mM MTX

MEM α	150 ml	150 ml
αFBS	7.5 ml	7.5 ml
G418 (100 mg/ml)	1.5 ml	1.5 ml
PBS	1.5 ml	1.5 ml
1 μ M MTX:	3 ml	1.5 ml

- remove the medium of the transfected cells in the 6 well dishes

- wash cells with PBS

- trypsinize cells with 500 μ l Trypsin (EDTA 5%)

- resuspend cells of each well in 45 ml selection medium
- seed cells of each well in 3 hybridoma dishes, 15 ml each hybridoma plate
- incubate cells for 2 weeks

12.7.95

Freezing of the CHO clones ST 10.1 (cells were transfected with the gene expression vector CHO1). VIGFS (5 μ M MTX) old master 1346, VIGF2 (100 μ M MTX), VIGF3 (100 μ M MTX)

ST 10.1: cells of 5 85-90% confluent T225 flasks were frozen in 50 1 ml aliquots 1×10^6 cells / aliquot

VIGFS (5 μ M MTX): cells of 1 85-90% confluent T225 flask was frozen in 10 aliquots of 1 ml 1×10^6 cells / aliquot

VIGF2 (100 μ M MTX): cells of 1 85-90% confluent T225 flask was frozen in 10 aliquots of 1 ml 1×10^6 cells / aliquot

VIGF3 (100 μ M MTX): cells of 1 85-90% confluent T225 flask was frozen in 10 aliquots of 1 ml 1×10^6 cells / aliquot

- ~~add~~ remove the medium of the T-flasks and wash the cells with 10 ml PBS (3-4 T225 flasks can be handled in parallel)
- add 5 ml Trypsin / EDTA and incubate 5' at 37°C
- add 6 ml MEM2 - Medium + 10% of FBS and resuspend cells in a final volume

Stably transfected CHO cells - CHO/TNF α

1.3.95

- Cells were transfected at 12.1.95 (see P.20) and seeded into hybridoma plates for selection (see p.21)

Observations:

- For all 3 constructs there were no clones found when cells were seeded in medium that contained 20mM MITX
- BUP1N346 = on three hybridoma plates there could only 1 clone be found.
(- clone was not transferred)

~~XXXXXX~~- TNF α (N346):Hybridoma
plate #

Clones #

1

2

2

13

3

6

- TNF α (CHO)

1

10

2

9

3

11

- The hybridoma cells with clones were labeled with a marker

- Tissue culture medium was removed and the plate was washed with 10ml PBS

- Remaining PBS was removed and at
- the wells that contained clones, ~~were~~ using a 20 μ l Pipet
 - ~~the wells that contained clones~~
 - 7 μ l of Trypsin was added to the wells that contained clones and cells were incubated for 5' at 37°C
 - cells of each well were transferred into the well of a 24 well dish
 - (1 ml MEM, 5% FBS, 1% PS, 20 mM HTX/well)

1996

Observation at growth of cells in 24 well dishes

TRF32	1.1	single cells	2.1	—
CHO	1.2	transfer cells into 6 well dish	2.2	—
20 mM HTX	1.3	→ 6 well dish	2.3	→ 6 well dish
	1.4	—	2.4	—
	1.5	S.C.	2.5	—
	1.6	→ 6 well dish	2.6	—
	1.7	→ 6 well dish	2.7	→ transfer cells to T25
	1.8	→ 6 well dish	2.8	S.C.
	1.9	→ 6 well dish	2.9	S.C.
	1.10	S.C.		
	3.1	—		
	3.2	—		
	3.3	S.C.		
	3.4	—		
	3.5	—		
	3.6	—		
	3.7	—		
	3.8	—		
	3.9	—		
	3.10	—		
	3.11	—		

TNF γ	1.1	S.C	2.6	-
U346	1.2	S.C	2.7	-
2mM MTX	1.3		2.8	-
	1.4		2.9	-
	2.1	-	2.10	-
	2.2	S.C	3.1	-
	2.3	S.C	3.2	-
	2.4	S.C	3.3	-
	2.5	-	3.4	-
	3.5	-		
	3.6	-		

CHO/DG44 cells: 1 vial was thawed and seeded
into a T75 flask in MEM 2⁺ medium, 5% DFBS,
1% PS

CHO/DG44 01.09.96 - 4

VIGF3: 1 vial was thawed and seeded into a T75 flask
(100 μ M) in MEM 2⁺ medium, 5% DFBS, 1% PS, 50 μ M MTX

VIGF3 01.09.96 - 0

VIGFS: 1 vial was thawed and seeded into a T75 flask
(5 μ M) in MEM 2⁺ medium, 5% DFBS, 1% PS, 5 μ M MTX

VIGFS 01.09.96 - 3

CHO/DG44 cells: passage cells 1:4 into
one T75 flask

1.12.96

CHO/DG44 01.09.96 - 5

VIGF3: passage cells 1:4 into 4 T75 flasks
(100 μ M)

VIGF3 01.09.96 - 1

VIGFS
(5 μ M)

passage cells 1:4 into one T75 flask

VIGFS 01.09.96 - 1

Markus work

(Markus - Sick)

1/15/96.

6:00 PM. CHO/dhfr

D644

(T-75)

1.11.96-5

CHO/dhfr

D644

(T-225)

1/15/96-6

all of culture pellet
 47.5 ml MEM- α + 1% PS (1/15/96)
 2.5 ml H-FBS (cat. 16000-044)
 (10+ ASP 4546)
 GIBCO

VIGFS T-75

1.9.96 (500 nM MTX)

VIGFS (T-225)

1/15/96 (500 nM MTX)

all of culture pellet
 50 ml MEM- α + 5% D-FBS + 1% PS
 with 500 nM MTX (1/15/96, 82)
 (5 ml of 500 nM MTX stock)

INF 2.7

T-75/CHO

(T-25)

20 nM

T-75/CHO

1/15/96

(20 nM)

all of culture pellet
 20 ml MEM- α + 5% D-FBS + 1% PS
 with 20 nM MTX (1/15/96, 82)
 (0.8 ml of 500 nM MTX)

500 nM MTX { 0.1 ml 500 nM MTX
 10 ml MEM- α + 5% D-FBS + 1% PS
 500 nM MTX { 0.1 ml 500 nM MTX
 10 ml MEM- α + 5% D-FBS + 1% PS

4 T 75 flasks VIGFS BIO96-1 submitted to
 June King

- ST5, ST10 cells: Comparative Study : 11.16.96

- to compare expression level after several passages
ST5 and ST10 cells were frozen and seeded
in MEM, 2%, 5% FBS, 1% PS, 80 μ M MTX

ST5 - 011096 - P0

ST10 - 011696 - P0

TNF α clones (cells transfected with CHO01 vector)

cells from 6 well dish, passage into T25 :

TNF α , CHO, 1.2

TNF α , CHO, 1.3

cells from 24 well dish, passage into 6 well dish :

TNF α , CHO, 1.10

TNF α , CHO, 2.2

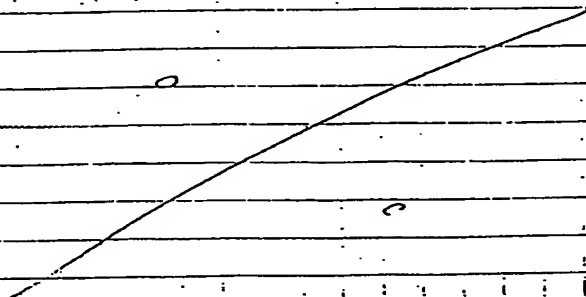
TNF α , CHO, 2.9

cells from 6 well dish passage into 6 well dish :

TNF α , CHO, 1.6

TNF α , CHO, 1.7

TNF α , CHO, 1.9



17.96

TNF γ - CHO cell lines transduced at 11.29.95
and transferred into selective medium at 12.1.95

- Several more clones were picked from hybridoma plates
into 24 well dishes:

TNF γ (cells were transduced with the CHO 1 vector)

- 1) TNF γ 4.1 / 112995 / PO1 to TNF γ 4.4 / 112995 / PO0
- 2) TNF γ 5.1 / 112995 / PO1 to TNF γ 5.10 / 112995 / PO1
- 3) TNF γ 6.1 / 112995 / PO1 to TNF γ 6.10 / 112995 / PO1

TNF γ (cells were transduced with the N396 vector)

- 4) TNF γ 4.1 / 112995 / PO1 to TNF γ 4.10 / 112995 / PO1
- 5) TNF γ 5.1 / 112995 / PO1 to TNF γ 5.4 / 112995 / PO1

- medium was removed from the hybridoma plates
- hybridoma plate was washed with 10 ml PBS
- PBS was removed
- the wells that were marked and numbered for harvest
before, were cleared from PBS using a 20 μ l Pipetman
and a index approx should yellow PB
- 5 wells were trypsinized at one time
(to control time of incub. with trypsin)
- 5' incubation at 37°C
- 10 μ l of medium was added to the cell
cells were transferred and seeded into the
well of a 24 well plate in

MEM + 5% dFBS (1% PS, 20 mM MTX
(using a 20 μ l Pipetman).

1.22.96 11 β Y-clones - Cells expanded in T25 flasks
for amplification and freezing of backups (MEM α , 5% dFBS, 1% PS
(CHO: cells were transfected with CHO1 derived vector) + 20 mM MTX)

- 1.) CHO/TNF γ 1.3 / 112995-P04 : 1 T25 confluent in 2 T25 flasks
- 2.) CHO/TNF γ 1.2 / 112995-P04 : 1 T25 confluent in 2 T25 flasks
- 3.) CHO/TNF γ 2.7 / 112995-P04 : 1 T25 confluent 1:10 in 1 T25 flask
1:10 in 1 T25 flask
- 4.) CHO/TNF γ 1.6 / 112995-P04 : 1.6w confluent in 2 T25 flasks
- 5.) CHO/TNF γ 1.7 / 112995-P04 : 1.6w confluent in 2 T25 flasks
- 6.) CHO/TNF γ 1.9 / 112995-P04 : 1.6w confluent in 2 T25 flasks
- 7.) CHO/TNF γ 2.3 / 112995-P03 : 1.6w confluent in 2 T25 flasks
- 8.) CHO/TNF γ 2.2 / 112995-P03 : 1.6w 40% confl. in 2 T25 flasks

Next steps with these clones:

- all clones split in 2 T25 flasks, one flask of each clone will be expanded into a T75 flask for freezing of 3.1 ml aliquots at 1×10^6 cells/ml. Cells of the second T25 flask are going to be seeded in 6 wells of a 6 well dish at 0, 20, 50, 100, 200, 500 mM MTX for amplification

1.23.96 Comparative Study ~~ST10.1 and CHO ST10.1~~ 346 ST5 and CHO ST10.1
in MEM α , 5% dFBS, 1% PS, 80 μ M MTX

- Friday 1.19.96

- ST5 011696-P00 \rightarrow P01 : 100% confluent T75 flask split 1:3 into 3 T75 flasks
- ST10.1 011696-P00 \rightarrow P01 : 100% confluent T75 flask split 1:3 into 3 T75 flasks

- 3 x 1ml aliquot of both clones were frozen at -80°C

- Tuesday 1.23.96

- T75 flasks are ca. 90% confluent, 1 T75 flask of each cell line was given to Marie Collins for long term comparison in spinner flasks

- 3 x 1ml aliquots of both cell lines were frozen at -80°C

- cells at 1 ST5 and 1 ST10.1 T25 flask were
passed into 2 T25 flasks at a split ratio of 1:3 → P2

Establishing of stable CHO-TNF α clones

1.25.96

(all clones were transfected with the CHO-O1 derived vector)

clones:	1) CHO/TNF α 1.3 / 112995-P04 → P05	2 confluent T25 flasks
	2) CHO/TNF α 1.2 / 112995-P04 → P05	2 confluent T25 flasks
	3) CHO/TNF α 2.7 / 112995-P04 → P05	1 confluent T25 flask 1 confluent T25 flask
	4) CHO/TNF α 1.7 / 112995-P04 → P05	2 confluent T25 flasks
	5) CHO/TNF α 1.9 / 112995-P04 → P05	2 confluent T25 flasks

all the clones:

first T25 flask: - take a 2 ml aliquot of the TCM
and freeze it in a cryovial at -20°C
for testing in a Western assay.

Freezing:

- trypsinise cells and resuspend in
16 ml final volume, seed 10 ml in (1:1.6)
T25 flask (MEM, 5% FBS, 1% PS, 20 mM MTX)
→ freeze these cells on Tuesday.
- cells of T25 TNF α 2.7, split 1:5 in T25

second T25 flask of every clone:

- trypsinise cells, and resuspend in 2 ml MEM, 5% FBS, 1% PS
- seed 1 ml in a T25 flask in MEM, 5% FBS, 1% PS, 20 mM MTX
next day replace medium with CHO-SFM-II + 1% PS
in order over WE and harvest samples for Western +
~~test~~ Dan Rodman's assays.

Testing:

Amplification:

- resuspend remaining 1 ml in a final volume of 6 ml
MEM, 5% FBS, 1% PS, 0 MTX

- distribute 1 ml cell suspension in each of a well of a 6 well plate

- add 0 / 50 μ l / 150 μ l / 250 μ l / 500 μ l and 1000 μ l of MEM, 5% dFBS, 1% PS, 800 nM MTX in order to achieve 0 / 20 nM / 60 nM / 100 nM / 200 nM and 400 nM MTX final concentrations in a final volume of 2 ml / well

- adjust final volumes to 2 ml / well with MEM, 5% dFBS, 1% PS, 0 MTX

1.26.96

Comparative Study 346 ST5 and CHO ST10.1:

1) - take 3 x 1 ml samples of each clone:

346 ST5 / 011696 - P02

CHO ST10.1 / 011696 - P02

freeze samples at -80°C

2) - passage cells 1:4 after WE. seed 2 flasks / clone

→ 346 ST5 / 011696 - P03

CHO ST10.1 / 011696 - P03

medium: MEM, 5% dFBS, 1% PS
800 nM MTX

CHO dhfr⁻ / DG 44 cells

Passage:

CHO dhfr⁻ DG 44 / 010996 - P08 → P09 1:10 seed 2 flasks

Medium: MEM, 5% dFBS, 1% PS,

Establishing of stable CHO-TNF γ -clones

- 1.) 1) CHO TNF γ 2.3 / 112995-P03 \rightarrow P04
 2) CHO TNF γ 2.9 / 112995-P03 \rightarrow P04
 3) CHO TNF γ 1.6 / 112995-P03 \rightarrow P04

- cells were confluent in 2 T25 flasks each clone
- split cells 1:5 and seed in 2 T25 flasks each clone in MEM α , 5% DFBS, 1% PS, 20mM MTK

- 2.) TNF γ clones growing in 6 well dishes, passage and seed cells in 6 well dishes: MEM α , 5% DFBS, 1% PS, 20mM MTK

- 1) CHO TNF γ 3.3 / 112995-P02-03 (3) CHO TNF γ 1.8 / 112995
 2) CHO TNF γ 1.10 / 112995-P02-03 medium was exchanged in 6 well dishes

- 3.) Clones in 24 well dishes, passage and seed cells in T25 or 6-well dishes: MEM α , 5% DFBS, 1% PS, 20mM MTK
 passage in 6 well dishes: passage in T25 dishes:

- | | |
|--|--|
| 1) CHO TNF γ 4.1 / 112995 P01 \rightarrow 02 | 1.) CHO TNF γ 6.4 / 112995 P01-02 |
| 2) CHO TNF γ 4.2 / 112995 P01 \rightarrow 02 | 2.) CHO TNF γ 6.7 / 112995 P01-02 |
| 3) CHO TNF γ 4.3 / 112995 P01 \rightarrow 02 | 3.) CHO TNF γ 6.8 / 112995 P01-02 |
| 4) CHO TNF γ 4.4 / 112995 P01 \rightarrow 02 | |
| 5) CHO TNF γ 5.5 / 112995 P01 \rightarrow 02 | |
| 6) CHO TNF γ 5.7 / 112995 P01 \rightarrow 02 | |
| 7) CHO TNF γ 5.9 / 112995 P01 \rightarrow 02 | |
| 8) CHO TNF γ 6.2 / 112995 P01 \rightarrow 02 | |
| 9) CHO TNF γ 6.10 / 112995 P01 \rightarrow 02 | |
| 10) CHO TNF γ 6.6 / 112995 P01 \rightarrow 02 | |

- 4.) N396 derived clones, cells grow in 24 well dishes, passage and seed cells into T25 flasks in MEM α , 5% DFBS, 1% PS

- 1) 396 TNF γ 2.3 / 112995 P01-02
 2) 396 TNF γ 4.1 / 112995 P01-02
 3) 396 TNF γ 4.3 / 112995 P01-02
 4) 396 TNF γ 4.4 / 112995 P01-02
 5) 396 TNF γ 4.5 / 112995 P01-02
 6) 396 TNF γ 4.6 / 112995 P01-02

~~1.27.96~~

- 7.) 346-TNF α 4.7 / 112995-P01-02
- 8.) 346-TNF α 4.10 / 112995-P01-02
- 9.) 346-TNF α 5.3 / 112995-P01-02
- 10.) 346-TNF α 5.4 / 112995-P01-02

1.27.96 Establishing of stable CHO-TNF α - clones

Clones for testing in Western blots and in ~~cell~~ Dan
Bednarik's assays:

- 1) CHO-TNF α 1.2 / 112995-P05
- 2) CHO-TNF α 1.3 / 112995-P05
- 3) CHO-TNF α 1.7 / 112995-P05
- 4) CHO-TNF α 1.9 / 112995-P05
- 5) CHO-TNF α 2.7 / 112995-P05

cells were seeded into 125 flasks on 1.25.96 (p. 45)

- Exchange the tissue culture medium

- remove medium
- wash twice with PBS to make sure to remove all traces of LPS
- add 5ml CHO-S-SFM II + 1% PS and incubate for 2 more days

- as negative control for the assays also change the medium to CHO-S-SFM II + 1% PS for clone #

ST 10.1 1011696-P03

1.29.96

- harvest supernatants of clones above and aliquot each sample in 5 x 1ml aliquots

- freeze down 3 aliquots / clone for westerns

- submit 2 x 1ml aliquot / clone to Dan Bednarik for antiviral assays

Establishing of Stable CHO-TNF α clones

1.30.96

T25 flasks confluent: \rightarrow split 50% \rightarrow T25 flasks, 25% \rightarrow T25 flasks
 25% \rightarrow 6 well amplification

(all clones transfected with transfected with pCHO-1
 (pC1) derived vector

- 1.) CHO TNF α 1.6 / 112995 - P04 - P05
- 2.) CHO TNF α 2.3 / 112995 - P04 - P05
- 3.) CHO TNF α 2.9 / 112995 - P04 - P05
- 4.) CHO TNF α 6.4 / 112995 - P02 - P03
- 5.) CHO TNF α 6.7 / 112995 - P02 - P03
- 6.) CHO TNF α 6.8 / 112995 - P02 - P03

all cell lines are
 confluent in T25 flask

- remove medium from flask
- wash cells with PBS
- trypsinise 500 μ l Trypsin 5' 37°C
- resuspend in 2 ml final volume MEM 1, 5% dFBS, 1% PS, 0.1% MTX

Freezing 1.) Seed 1 ml of the cell susp. in a T25 flask in 20 ml
 MEM 1, 5% dFBS, 1% PS, 20 mM MTX to expand
 cells for freezing of selected clones

Amplification 2.) Seed 0.5 ml in 5 ml MEM 1, 5% dFBS, 1% PS, 0.1% MTX
 into the first well of a 6 well plate, mix well and
 seed 1 ml of the cell suspension into each well of
 the 6 well plate

- add 0.150 μ l / 150 μ l / 250 μ l / 500 μ l / 1000 μ l
 of MEM 1, 5% dFBS, 1% PS, 800 mM MTX
- adjust volume to 2 ml each well

\Rightarrow MTX end concentrations 0, 20 mM, 60 mM, 100 mM,
 200 mM, 400 mM

Testing 3.) Seed 0.5 ml of cell suspension in T25 flask in
 MEM 1, 20 mM MTX, 5% dFBS, 1% PS

- Change Med. when cells are confluent to CHO-S-Selectin 1% PS

- incubate for 2 more days, then harvest medium for testing in Western + initial assay

Establishing of stable CHO-TNF δ clones (pCI-constructs)

1.30.96 freezing of inprocess cells after clonal selection in hybridoma plates, expanding of clones in 24 well, 6 well, T25 and T75 flasks in presence of 20mM MTX

(continued from p. 95)

- 1) CHO-TNF δ 1.2/112995-P05-06
- 2) CHO-TNF δ 1.3/112995-P05-06
- 3) CHO-TNF δ 1.7/112995-P05-06
- 4) CHO-TNF δ 1.9/112995-P05-06
- 5) CHO-TNF δ 2.7/112995-P05-06

20mM MTX

- hypoxinize cells and resuspend in 10ml
- spin 10' at 800 rpm
- resuspend cells in 3ml freezing medium:

MEM α , 5% FBS, 10% DMSO

- freeze down 3 x 1ml aliquots of each clone
- store in Nalgene freezing box at -80°C over night
- transfer cells into the -140°C freezer

1.30.96 CHO-TNF δ clones: expanding cells for freezing

cells are confluent in well of a 6 well dish:

passage cells into a T25 flask in

MEM α , 5% FBS, 1% PS, 20mM MTX

- 1) CHO-TNF δ 3.3/112995-P03-04
- 2) CHO-TNF δ 4.0/112995-P03-04
- 3) CHO-TNF δ 6.2/112995-P02-03
- 4) CHO-TNF δ 6.6/112995-P02-03
- 5) CHO-TNF δ 6.10/112995-P02-03

Comparative Study 346 ST5 and CHO ST 10.1

1.30.96

Take 3 x 1ml aliquots of each clone and passage
the cells in MEM, 5% FBS, 1% PS, 80% 4 HX
split into 1-3

1) 346 ST5 / 011696-P03-04

2) CHO ST 10.1 / 011696-P03-04

CHO cells / DG44 cells

1.30.96

Passage

CHO cells / DG44 / 010996-P09-10

split 1:10 seed 2 flasks

MEM, 5% FBS, 1% PS

Establishing of stable CHO-TNF α clones

1.31.96

Change of medium for testing Westerns and in
antigenic assays.

1.) CHO TNF α 1.6 / 112995-P05

2.) CHO TNF α 2.3 / 112995-P05

3.) CHO TNF α 2.9 / 112995-P05

4.) CHO TNF α 6.4 / 112995-P03

5.) CHO TNF α 6.7 / 112995-P03

6.) CHO TNF α 8.8 / 112995-P03

T25 flasks 90% confluent

- remove TC-medium

- wash 2 x with PBS

- add 5ml CHO-S-SFM + 1% PS

- incubate for 2 more days

1.31.96 Establishing of CHO TNF γ clones

Amplification: continued from p.45

cells confluent at 60 mM MTX, decreasing to 20 μ M [MTX]

- 1.) CHO TNF γ 1.2 / 112996 - P05 \rightarrow O6
- 2.) CHO TNF γ 1.3 / 112996 - P05 \rightarrow O6
- 3.) CHO TNF γ 1.7 / 112996 - P05 \rightarrow O6
- 4.) CHO TNF γ 1.9 / 112996 - P05 \rightarrow O6
- 5.) CHO TNF γ 2.7 / 112996 - P05 \rightarrow O6

- remove TCM, wash with PBS (1 ml / well)
- trypsinise 5', 500 μ l Trypsin / EDTA the wells with ~~200~~, ~~400~~, 60, 100, 200, 400 mM MTX
- add 1 ml MEM, 5% dFBS, 1% PS, 0 MTX to each of the four wells
- pool the cells at the 4 wells in 6 ml final volume
- seed the 6 ml cell susp. into 6 wells of a 6-w. dish at 1 ml / well
- add 0, 50, 150, 250, 500 and 1000 μ l of MEM, 5% dFBS, 1% PS, 800 mM MTX to achieve 0, 20 mM, 60 mM, 100 mM, 200 mM, 400 mM [MTX].
- add MEM, 5% dFBS, 1% PS, 0 MTX to a final volume of 2 ml / well of the 6 well dish.

1.31.96 Amplification of VIBF 5 up to 100 μ M MTX continued from P.43:

- several clones / well from 20 to 100 μ M MTX
- remove medium, trypsinise 5' 200 μ l / well, resuspend cells in 6 ml final volume in MEM, 5% dFBS, 1% PS, 0 MTX

- Seed cells in 6 wells of a 6-w. dish, 1 ml/cell
- add 0, 100 μ l, 200 μ l, 500 μ l, 800 μ l, 1000 μ l at 200 μ M MTX to achieve 0, 10 μ M, 20 μ M, 50 μ M, 80 μ M and 100 μ M MTX (MEM, 5% dFBS, 1% PS, 200 μ M MTX)
- aspirate wells to 2 ml final volume (well (MEM, 5% dFBS, 1% PS, 0 MTX))

Establishing of stable CHO-TNF γ clones (p1 const.) 2.1.96.

freezing of in process cells after clonal selection and expanding of clones in T75 flasks in presence of 20 μ M MTX.

(Continued from p. 49)

- 1.) CHO-TNF γ 1.6 / 112995-p06 \rightarrow 07
- 2.) CHO-TNF γ 6.4 / 112995-p03 \rightarrow 04
- 3.) CHO-TNF γ 6.7 / 112995-p03 \rightarrow 04
- 4.) CHO-TNF γ 6.8 / 112995-p03 \rightarrow 04

(20 μ M MTX)

- trypsinize cells and resusp. in 1 ml
- spin 10' at 800 RPM
- resuspend in 3 ml freezing medium (MEM, 5% dFBS, 10% DMSO)
- freeze down 3 ml aliquots of each clone
- freeze in Nalgene freezing box at -80°C overnight
- transfer cells in -140°C freezer

Establishing stable 346-TNF γ clones (pN346 construct)

expanding of clones for freezing (backups)
clones growing in 24 well dishes (see p. 42)

- 1.) 346-TNF γ 4.2 / 112995-p01-02
- 2.) 346-TNF γ 4.8 / 112995-p01-02
- 3.) 346-TNF γ 4.9 / 112995-p01-02
- 4.) 346-TNF γ 5.1 / 112995-p01-02
- 5.) 346-TNF γ 5.2 / 112995-p01-02

- remove TCM, trypsinize
200 μ l 5'

- resusp. in 2 ml MEM, 5% dFBS
1% PS, 20 μ M MTX

- seed in 6 well

2.1.96 Establishing stable 346 TNF α clones (pN346 derived)

Several more clones were picked from hybridoma pools into 24 well dishes:

- 1.) 346 TNF α 6.1 / 112995-p01
- 2.) 346 TNF α 6.2 / 112995-p01
- 3.) 346 TNF α 6.3 / 112995-p01
- 4.) 346 TNF α 6.4 / 112995-p01
- 5.) 346 TNF α 6.5 / 112995-p01
- 6.) 346 TNF α 6.6 / 112995-p01
- 7.) 346 TNF α 6.7 / 112995-p01
- 8.) 346 TNF α 6.8 / 112995-p01
- 9.) 346 TNF α 6.9 / 112995-p01
- 10.) 346 TNF α 6.10 / 112995-p01

Steps see protocol p. 42

Establishing stable CHO TNF α clones (pCI derived)

clones that were growing in 6 well dishes or 24 well dishes were passaged into 6 well dishes in MEM 2, 5% DMS, 20 mM MTX 17. PS (Cells are going to be expanded into T25 flasks for freezing of backup clones)

- 1.) CHO TNF α 5.1 / 112995-p01-02
- 2.) CHO TNF α 5.2 / 112995-p01-02
- 3.) CHO TNF α 5.3 / 112995-p01-02
- 4.) CHO TNF α 5.4 / 112995-p01-02
- 5.) CHO TNF α 5.6 / 112995-p01-02
- 6.) CHO TNF α 5.10 / 112995-p01-02
- 7.) CHO TNF α 6.1 / 112995-p01-02
- 8.) CHO TNF α 6.5 / 112995-p01-02
- 9.) CHO TNF α 4.3 / 112995-p02-03
- 10.) CHO TNF α 5.7 / 112995-p02-03

} from 24 well

} from 6 well

2.1.96

clones that were growing in 6 well dishes were passaged into T25 or T75 flasks in MEM, 5% FBS, 1% PS, 20mM NTK (expand cells in T75 flasks for freezing of backup clones) 2.1.96

- 1.) CHO TNE γ 4.1 / 112995-p02-03 \rightarrow T25
- 2.) CHO TNE γ 4.2 / 112995-p02-03 \rightarrow T25
- 3.) CHO TNE γ 4.4 / 112995-p02-03 \rightarrow T25
- 4.) CHO TNE γ 5.5 / 112995-p02-03 \rightarrow T25
- 5.) CHO TNE γ 5.9 / 112995-p02-03 \rightarrow T25

Establishing of stable CHO clones TNE γ (pCI const.) 2.2.96

freezing of in process cells after clonal selection and expansion of clones into T25 flasks in presence of 20mM NTK.

(continued from p 45. 2.1.96)

- 1.) CHO TNE γ 2.3 / 112995-p06
- 2.) CHO TNE γ 2.9 / 112995-p06
- 3.) CHO TNE γ 3.3 / 112995-p05
- 4.) CHO TNE γ 6.2 / 112995-p04
- 5.) CHO TNE γ 6.6 / 112995-p04
- 6.) CHO TNE γ 6.10 / 112995-p04

- trypsinise cells and resusp. in 10ml
- spin 10' at 800 rpm, resusp. cells in 3ml freezing medium (MEM, 5% FBS, 10% DMSO)
- freeze down in 3x 1ml aliquot and store in Helgene freezing box at -80°C over night
- transfer cells in -140°C freezer

2.1.96

2.2.96 Establishing of stable CHO TNF α clones

harvest of medium for testing in western- and
antiviral assays (continued from p. 51)

- after 2 days of incubation in CHO-S-SFM-II + 1% PS
the medium was harvested and frozen in
5 x 1 ml aliquots each done at -20°C

- 1.) CHO TNF α 1.6 / 112995 p 5
- 2.) CHO TNF α 2.3 / 112995 p 5
- 3.) CHO TNF α 2.9 / 112995 p 5
- 4.) CHO TNF α 6.4 / 112995 p 3
- 5.) CHO TNF α 6.7 / 112995 p 3
- 6.) CHO TNF α 6.8 / 112995 p 3

2.2.96 Comparative study CHO ST 10.1 / 346 ST 5

take 3 x 1 ml aliquots each clone and passage
the cells in MEM $^{+}$, 5% dFBS, 1% PS, 80% MEM

- 1.) 346 ST 5 / 011696 - p 04-05 split 1:4
- 2.) CHO ST 10.1 / 011696 - p 04-05 split 1:4

Passaging of CHO dhr- / DR44 cells

CHO dhr- DR44 / 010996 - p 11 split 1:10

seed me T225 flask in MEM $^{+}$, 5% dFBS, 1% PS

2.2.96

Establishing of stable CHO TNE & clones

2.5.96

Freezing of clones (backup), in processables after clonal selection, and expanding in T25 flasks at 20 mM MTK

- 1.) CHO TNE #1.10 / 112995 - p05
- 2.) CHO TNE #5.5 / 112995 - p04
- 3.) CHO TNE #5.9 / 112995 - p04

Comparative Study CHO ST10.1 - 346 ST5

2.5.96

Freezing of clones for further analysis

- 1.) 346 ST5 / 011696 - p05-06
- 2.) CHO ST10.1 / 011696 - p05-06

Passaging of CHO cells / DS44

2.6.96

CHO cells DS44 / 010996 - p012 Split 1:10

Seed one T25 flask in MEM 1⁺, 5% dFBS, 1% PS

Comparative Study CHO ST10.1 - 346 ST5

2.6.96

Take 3 x 1ml aliquots each clone and passage the cells in MEM 1⁺, 5% dFBS, 1% PS, 80 μ M MTK

- 1.) 346 ST5 / 011696 - p05-06 Split 1:3
- 2.) CHO ST10.1 / 011696 - p05-06 Split 1:3

2.6.26 Establishing of stable CHO TNF γ

Expanding of cells for freezing, passage cells from 6 wells to T75 flasks in 20 mM MTX (- freezing backups only)

- 1.) CHO TNF γ 6.5 / 112995 - p02-03
- 2.) CHO TNF γ 4.3 / 112995 - p03-04
- 3.) CHO TNF γ 5.7 / 112995 - p03-04
- 4.) CHO TNF γ 5.4 / 112995 - p02-03

Expanding of cells for freezing, passage cells from T25 to T75 flasks in 20 mM MTX (freezing backups only)

- 1.) CHO TNF γ 4.1 / 112995 - p03-04
- 2.) CHO TNF γ 4.2 / 112995 - p03-04
- 3.) CHO TNF γ 4.4 / 112995 - p03-04

Establishing of stable CHO TNF γ clones

Amplification: continued from page 52

cells confluent at 200 mM and low cells at 400 mM

- 1.) CHO TNF γ 1.2 / 112995 - p06-02
- 2.) CHO TNF γ 1.3 / 112995 - p06-02
- 3.) CHO TNF γ 1.2 / 112995 - p06-02
- 4.) CHO TNF γ 1.9 / 112995 - p06-02
- 5.) CHO TNF γ 2.7 / 112995 - p06-02

- remove TCM cells with 1 ml PBS/well
 trypsinise 5' 200 μ l Trypsin/EDTA/well
 (only 200 mM and 400 mM well)

- pool cells of 2 wells and resuspend in 6 ml FV in MEM 2, 5% ABFS, 1% PS, 0.1 MTX

- seed the cells into 6 wells of a new 6 well dish, 1 ml/well

8.801

- add 0, 100, 200, 500, and 1000 μ l of ~~MEM~~ MEM, 5% dFBS, 1% PS, 2 μ M MTX to the wells in order to achieve 0, 100 nM, 200 nM, 500 nM, 800 nM and 1 μ M MTX final concentration

- add MEM, 5% dFBS, 1% PS, 0 MTX, adjust final volume to 2 ml / well

Establishing of stable CHO TNF α clones

2.6.96

Amplification - continued from p. 49

cells grow in 6 wells 20 - 400 nM MTX

1.) CHO TNF α 1.6 / 112995 - P05-05	pool	100, 200, 400 nM
2.) CHO TNF α 2.3 / 112995 - P05-06	pool	20, 40, 100, 200, 400 nM
3.) CHO TNF α 2.9 / 112995 - P05-06	pool	60, 100, 200, 400 nM
4.) CHO TNF α 6.4 / 112995 - P03-04	pool	200, 400 nM
5.) CHO TNF α 6.7 / 112995 - P03-04	pool	100, 200, 400 nM
6.) CHO TNF α 6.8 / 112995 - P03-04	pool	200, 400 nM

- remove TCM wash with 1 ml PBS / well

- trypsinise 5', 200 μ l Trypsin EDTA / well (wells that are pooled only)

- pool cells and resusp. in 6 ml final volume in MEM, 5% dFBS, 1% PS, 0 MTX

- seed cells in 6 wells at 2.6 cells / well 1 ml / well

- add 0, 50, 150, 250, 500, 1000 μ l of MEM, 5% dFBS, 1% PS, 0.8 μ M MTX to achieve 0, 20, 60, 100, 200, 400 nM MTX final concentration

- add MEM, 5% dFBS, 1% PS, 0 MTX adjust final volume to 2 ml / well

2.7.96

Establishing of CHO TNE⁺ clones

Submitted samples for testing in antiviral assays.

- 1.) CHO TNE⁺ 1.6 / 112995 p5
- 2.) CHO TNE⁺ 2.3 / 112995 p5
- 3.) CHO TNE⁺ 2.9 / 112995 p5
- 4.) CHO TNE⁺ 6.4 / 112995 p5
- 5.) CHO TNE⁺ 6.7 / 112995 p5
- 6.) CHO TNE⁺ 6.8 / 112995 p5

Samples that were generated on 2.2.96 (p56), were submitted to Dan Bednarek for testing for antiviral activity.

2.8.96

Establishing of stable CHO TNE⁺ clones

Preparation of samples for Western analysis:

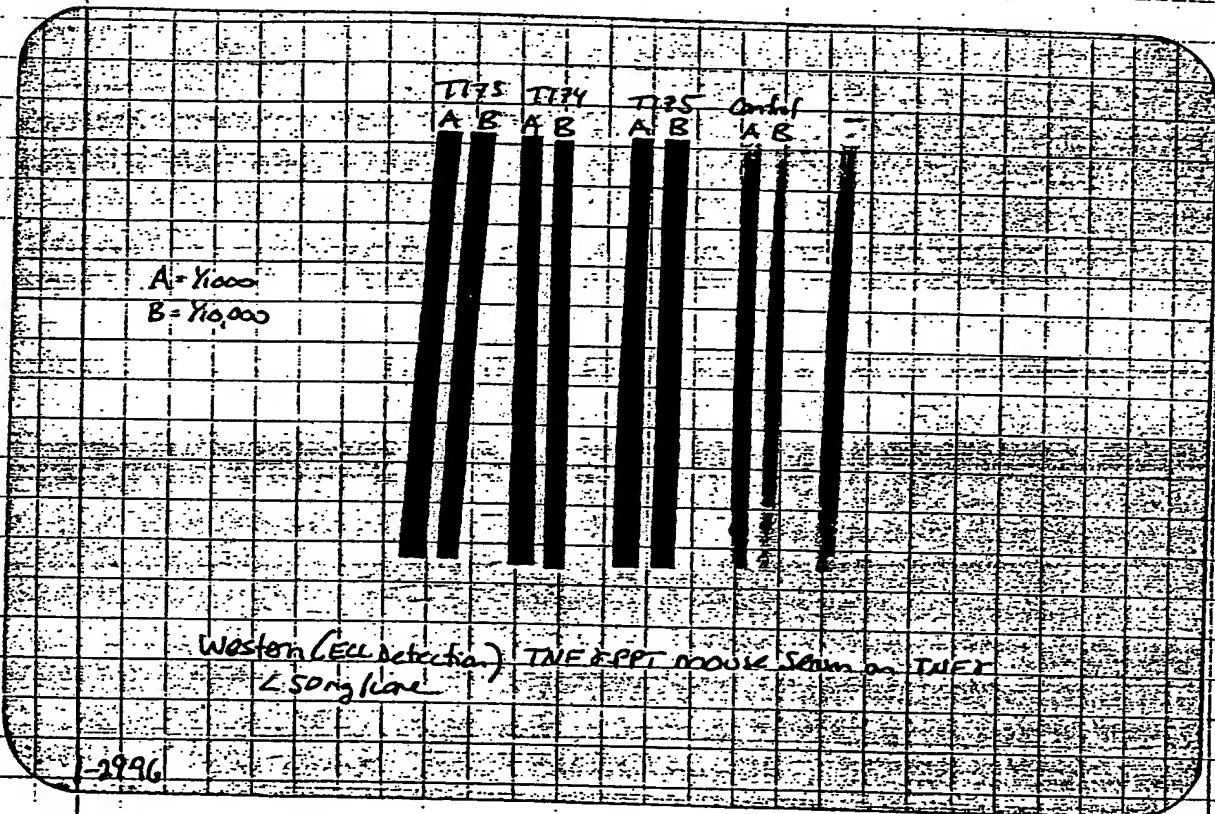
- all clones were seeded into 25 cm² T flasks for dosing see p. 45 and p. 49
 - after over night incubation the medium was replaced by CHO-S-SFM II + 1% Serum (CBFS)
 - cells were incubated for 2 more days before supernatants were harvested and frozen at -20°C.
- see p. 48, p. 56

Freezer Box1-1

In Process Cells							
Recombinant Cell Line:				CHO/pC1/TNF γ IL6			
Date:				02.07.96			
Clone ID:				HLTBT71			
Project Code:				HG10700			
Rack Number:		1					
Shelf Number:		1					
Box Number:		1,1					
Freezer:				Equipment Room/BPEC			
Host Cell Line:				CHO dhfr- (ATCC)			
Passage Number Host Cells:				-			
Expression vector:				pC1/TNF γ IL6			
Construct made by:				Guo-Liang Yu			
Date of Transfection:				11.29.95			
Cell Line Established by:				Markus Buergin			
Notebook:				443			
	Clone Number	Passage Number	[MTX]	Freezing Date	Location (in box)	Tested	Amplification
1	CHO TNF γ 1.2	112995-P06	20nM	01.30.96	A1-3	AV	+
2	CHO TNF γ 1.3	112995-P06	20nM	01.30.96	B1-3	AV	+
3	CHO TNF γ 1.7	112995-P06	20nM	01.30.96	C1-3	AV	+
4	CHO TNF γ 1.9	112995-P06	20nM	01.30.96	D1-3	AV	+
5	CHO TNF γ 2.7	112995-P06	20nM	01.30.96	E1-3	AV	+
6	CHO TNF γ 1.6	112995-P07	20nM	02.01.96	F1-3	AV	+
7	CHO TNF γ 6.4	112995-P04	20nM	02.01.96	G1-3	AV	+
8	CHO TNF γ 6.7	112995-P04	20nM	02.01.96	H1-3	AV	+
9	CHO TNF γ 6.8	112995-P04	20nM	02.01.96	A4-6	AV	+
10	CHO TNF γ 2.3	112995-P06	20nM	02.02.96	B4-6	AV	+
11	CHO TNF γ 2.9	112995-P06	20nM	02.02.96	C4-6	AV	+
12	CHO TNF γ 3.3	112995-P05	20nM	02.02.96	D4-6		
13	CHO TNF γ 6.2	112995-P04	20nM	02.02.96	E4-6		
14	CHO TNF γ 6.6	112995-P04	20nM	02.02.96	F4-6		
15	CHO TNF γ 6.10	112995-P04	20nM	02.02.96	G4-6		
16	CHO TNF γ 1.10	112995-P05	20nM	02.05.96	H4-6		
17	CHO TNF γ 5.5	112995-P04	20nM	02.05.96	A8-10		
18	CHO TNF γ 5.9	112995-P04	20nM	02.05.96	B8-10		
19							
20							
22							
23							
24							
25							

Immunoprecipitation with TNF γ - Mouse - Serum

Western with TNF γ - PPT - Mouse Serum by Ellie Bonfanti



Samples that were Immuno Precipitated:

- 1.) CHO SFM 4 - 1% α FB5
- 2.) ST 10.1 / 011696 - P03
- 3.) CHO TNF γ 1.2
- 4.) CHO TNF γ 1.3
- 5.) CHO TNF γ 1.7
- 6.) CHO TNF γ 1.9
- 7.) CHO TNF γ 2.7
- 8.) CHO TNF γ 1.6
- 9.) CHO TNF γ 6.4
- 10.) CHO TNF γ 6.7

- (1.) CHO TNF α 6.8
 (2.) CHO TNF α 2.3
 (3.) CHO TNF α 2.9

- 1.) dilute mouse anti-rat IgG (2 μ l in 18 μ l PBS) (T175)
- 2.) add 1 μ l of the 1:10 AB dilution to 500 μ l CHO cell supernatant
- 3.) incubate 30', RT, on rotator
- 4.) wash Protein G (Gamma bind Plus Sepharose Pharmacia Code No. 17-0886-01)

- spin 1ml of Protein G, 5 sec at 10000 rpm
- aspirate sup, wash with PBS
- repeat 1x
- resuspend Protein G in PBS 10:1: BSA

- 5.) add 20 μ l Protein G to sample
- 6.) incubate for 30' RT, rotator
- 7.) spin 10-20 sec at 10K RPM
- aspirate supernatant
- 8.) wash 3x with 1ml PBS
- spin, remove supernatant
- 9.) add 20 μ l 2x sample buffer, boil 10'
- spin after boiling
- 10.) Transfer supernatant into new Eppendorf tube
- dialyze at -20°C

(\rightarrow load samples on gel)

2.9.96 Comparative study ST5, ST10.1

take 3 x 1ml aliquots from each clone and
passage in MEM 2⁻, 5% dFBS, 1% PS, 80 μ M MTX
Split 1:3

- | | | |
|----|-------------------------|---------------------|
| 1) | 346 ST5 / 011696 p07 | (2 T 25 passages) |
| 2) | CHO ST10.1 / 011696 p02 | (2 T 25 passages) |

2.9.96 CHO d4R^r - DQ44

CHO d4R^r / DQ44 / 010996 - P13 split 1:8

passage into MEM 2⁻, 5% dFBS, 1% PS

2.9.96 Establishing of stable 346-VIGFS cells

amplification up to 80 μ M MTX:
(continued from P52)

cells grown in 6 wells at 50 μ M, 80 μ M, 100 μ M MTX

pool cells growing at the concentrations 50 μ M, 80 μ M, 100 μ M
and seed into a T 75 flask in 80 μ M MTX
(MEM 2⁻, 5% dFBS, 1% PS)

2.9.96 Establishing of stable CHO TWF 2 clones

freezing of backup in process cells:

- 1.) CHO TWF 2 5.7 / 112995 - p05
- 2.) CHO TWF 2 5.4 / 112995 - p04
- 3.) CHO TWF 2 4.2 / 112995 - p05
- 4.) CHO TWF 2 6.5 / 112995 - p04
- 5.) CHO TWF 2 4.1 / 112995 - p05

Establishing stable 346 TNF α clones:

expanding of backup clones: (for freezing)

6 well: seed: 53

24 well: seed: 59

- replace the medium with MEM, 5% dFBS, 1% PS, 20 mM MTX

Establishing of stable 346 TNF α clones:

split confluent T25 flasks:

50% seed in T25 flask in MEM, 5% dFBS, 1% PS, 20 mM MTX for freezing

25% seed in T25 flask in MEM, 5% dFBS, 1% PS, 20 mM MTX for testing, replace medium with CHO-S-SFM-C + 1% Serum + 1% PS tomorrow

25% seed in 6 wells of a 6 well dish for application of MTX concentrations:

0, 50, 100, 150, 250, 500 mM MTX

(\rightarrow 1 μ M-MTX stock solution!)

- 1) 346 TNF α 2.3 / 112995 - p03
- 2) 346 TNF α 4.1 / 112995 - p03
- 3) 346 TNF α 4.3 / 112995 - p03
- 4) 346 TNF α 4.4 / 112995 - p03
- 5) 346 TNF α 4.5 / 112995 - p03
- 6) 346 TNF α 4.6 / 112995 - p03
- 7) 346 TNF α 4.7 / 112995 - p03
- 8) 346 TNF α 4.8 / 112995 - p03
- 9) 346 TNF α 5.3 / 112995 - p03
- 10) 346 TNF α 5.4 / 112995 - p03

Establishing stable CHO-TNF γ clones:

2.9.96 Proteingel and Electrophoretic transfer for Western assays:

(continued from p. 63)

- The supernatants were concentrated by immunoprecipitation and prepared like described on p. 60-63

- run 2 proteingels: 4-20% NOVEX
68 ~~mA~~ mA
1 h

Gel 1:

1.)	M	(NOVEX prest. LM)
2.)	1.2	CHO-TNF γ
3.)	1.3	-II-
4.)	1.7	-II-
5.)	1.9	-II-
6.)	2.7	-II-
7.)	1.6	-II-
8.)	neg control (ST10.1)	
9.)	pos control 50ng TNF γ	
10.)	M	

Gel 2:

1.)	M
2.)	6.4 CHO-TNF γ
3.)	6.7 -II-
4.)	6.8 -II-
5.)	2.3 -II-
6.)	2.9 -II-
7.)	pos control 50ng TNF γ
8.)	M
9.)	M
10.)	neg control (CHO-S-FM β)

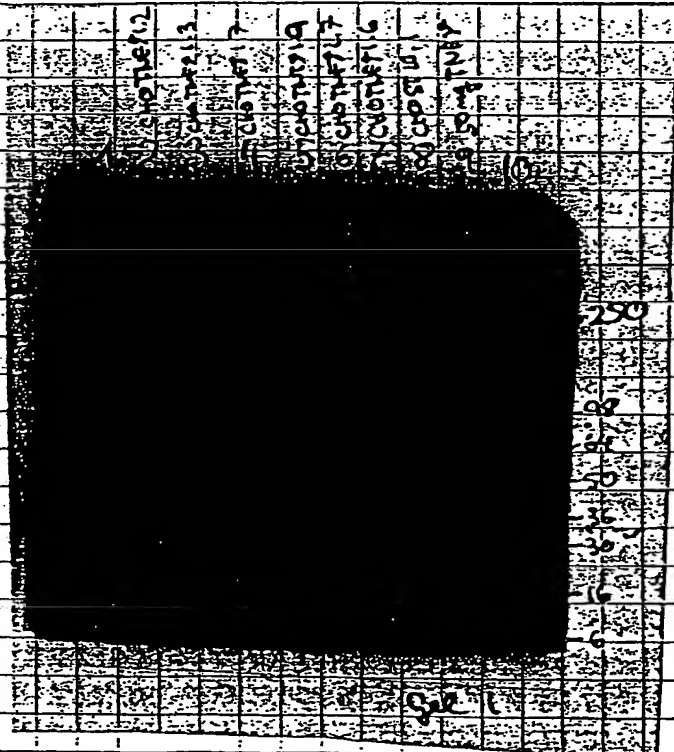
Western blot: Novex nitrocellulose membrane

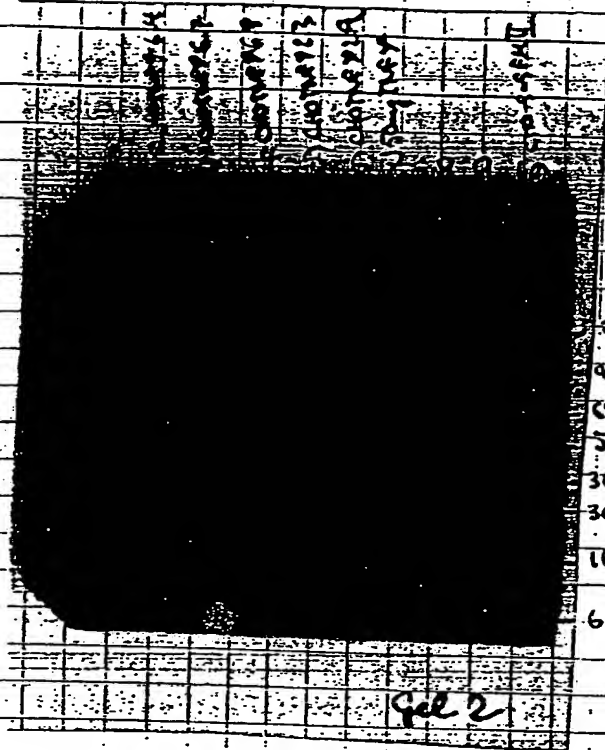
2.12.96

- prewet in blocking buffer

- electrotransfer at 30V, 1h
- 1h blocking 4% nonfat milk
- 2 x 10' wash with 1 x PBS
- 2.5h incubation with 1st AB. (Mouse-anti TNF α -PPT
Ab dil. 1:5000 * T.175 see p. 62)

- rinse 3 x with 1 x PBS, wash 3 x 10' with 1 x PBS
- 1h incubation with 2nd AB 1:2000 (anti mouse AB-APC)
- rinse 3 x with 1 x PBS, wash 3 x 10' with 1 x PBS
- 1' detection ECL
- 1 sec. exposure





2.12.96

Establishing of stable 346 TME clones

Change medium to CHO-S-SFM II + (1:10) PS of the cells in T25 flasks for testing (see p. 65)

- remove the TCM
- wash 2 x with PBS
- add 5ml of CHO-S-SFM II + (1:10) PS to each T25 flask

- 1.) 346 TME 2.3 / 112995 p03
- 2.) 346 TME 4.1 / 112995 p03
- 3.) 346 TME 4.3 / 112995 p03
- 4.) 346 TME 4.4 / 112995 p03
- 5.) 346 TME 4.5 / 112995 p03
- 6.) 346 TME 4.6 / 112995 p03
- 7.) 346 TME 4.7 / 112995 p03
- 8.) 346 TME 4.10 / 112995 p03
- 9.) 346 TME 5.3 / 112995 p07
- 10.) 346 TME 5.9 / 112995 p03

CHO α tko- DG44 / 010996 p03-14

2.12.95

- passage cells. Split ratio 1:10 and prepare cells for harvesting
- resuspend 3ml of the cell susp. in 11.5 ml MEM L⁺, 5% FBS, 1% PS
- seed 1ml of the cell suspension into each of 12 wells of a 2 6-well dishes (1:45 dil)
- cells for harvesting

Comparative Study 346 ST5 - CHO ST10.1

2.12.95

Take 3x1ml aliquots from each clone and passage in
MEM L⁺, 5% FBS, 1% PS, 80 μ M MTX

- 1.) 346 ST5 / 011696 p08 (split ratio 1:4)
- 2.) CHO ST10.1 / 011696 p08 (split ratio 1:2)

Establishing of stable 346 TNF α clones and CHO TNF α clones 2.13.96

Freezing of in process cells after clonal selection, expanding
of clones into T75 flasks (see p. 65)

- 1.) 346 TNF α 2.3 / 112995 p03
- 2.) 346 TNF α 4.1 / 112995 p03
- 3.) 346 TNF α 4.3 / 112995 p03
- 4.) 346 TNF α 4.4 / 112995 p03
- 5.) 346 TNF α 4.5 / 112995 p03
- 6.) 346 TNF α 4.6 / 112995 p03
- 7.) 346 TNF α 4.7 / 112995 p03
- 8.) 346 TNF α 4.10 / 112995 p03
- 9.) 346 TNF α 5.3 / 112995 p03
- 10.) 346 TNF α 5.4 / 112995 p03
- 11.) CHO TNF α 4.3 / 112995 p04
- 12.) CHO TNF α 4.4 / 112995 p04

2.14.96 Establishing stable TNF α clones
(346TNF α derived from pN346 vector)

- Harvested TCM from T25 flasks (SFH + 1% PS)
for testing in Western blots and anti-viral assays

- 1.) 346TNF α 2.3 / 112995 p03 (5 x 1ml)
- 2.) 346TNF α 4.1 / 112995 p03 (5 x 1ml)
- 3.) 346TNF α 4.3 / 112995 p03 (5 x 1ml)
- 4.) 346TNF α 4.4 / 112995 p03 (5 x 1ml)
- 5.) 346TNF α 4.5 / 112995 p03 (5 x 1ml)
- 6.) 346TNF α 4.6 / 112995 p03 (5 x 1ml)
- 7.) 346TNF α 4.7 / 112995 p03 (3 x 1ml)
- 8.) 346TNF α 4.10 / 112995 p03 (5 x 1ml)
- 9.) 346TNF α 5.3 / 112995 p03 (4 x 1ml)
- 10.) 346TNF α 5.4 / 112995 p03 (4 x 1ml)

- Harvested 3, 4 or 5 x 1ml aliquots

- Submitted 2 x 1ml aliquots of each clone
to Dan Bednarek for testing in anti-viral assays
(except clone 346TNF α 4.7 submitted only 1 x 1ml)

- Freeze remaining 1ml aliquots for testing in
Western blots

2.14.96 Establishing stable TNF α clones (346TNF α derived from pN346)

- expanding of backup clones from 4 6 well or
24 well plates into T25 flasks
→ expand cells for freezing of backup clones
in MEM + 20% FBS + 5% DMSO, 1% PS
from 6 well dishes:

- 1.) 346TNF α 4.2 / 112995 p03
- 2.) 346TNF α 4.8 / 112995 p03
- 3.) 346TNF α 4.9 / 112995 p03
- 4.) 346TNF α 5.1 / 112995 p03
- 5.) 346TNF α 5.2 / 112995 p03

from 24 well dishes

- 6.) 346TNF γ 6.1 / p02 CM
- 7.) 346TNF γ 6.2 / p02 N⁺
- 8.) 346TNF γ 6.3 / p02
- 9.) 346TNF γ 6.4 / p02 CM⁺
- 10.) 346TNF γ 6.5 / p02 X⁺
- 11.) 346TNF γ 6.6 / p02
- 12.) 346TNF γ 6.7 / p02
- 13.) 346TNF γ 6.8 / p02
- 14.) 346TNF γ 6.9 / p02
- 15.) 346TNF γ 6.10 / p02

Establishing of stable CHO TNF γ clones

2.15.96

Amplification: continued from p 58

cells growing at 0-1000 mM NaX pooled and seeded at 0, 100, 200, 500, 800 and 1000 mM NaX

- 1.) CHO TNF γ 1.2 / U2995 p08
- 2.) CHO TNF γ 1.3 / U2995 p08
- 3.) CHO TNF γ 1.7 / U2995 p08
- 4.) CHO TNF γ 1.9 / U2995 p08
- 5.) CHO TNF γ 2.2 / U2995 p08

cells growing at 0-400 mM NaX pooled and seeded at 0, 50, 100, 150, 250, 500 mM NaX

- 1.) CHO TNF γ 1.6 / U2995 p02
- 2.) CHO TNF γ 2.3 / U2995 p02
- 3.) CHO TNF γ 2.4 / U2995 p02
- 4.) CHO TNF γ 6.8 / U2995 p08

- 5.) CHO TNF γ 6.9 / U2995 p06
- 6.) CHO TNF γ 6.2 / U2995 p06

exchange TCM in old cells, same [NaX] lit. allow clones (1-9)

2.15.96 Establishing of stable 346 TNF α clones

Amplification continued from p 65

cells grow in wells from 0-500 μ M MTX
pick cells and seed in 6 wells at a 6 well dish
at 0, 50, 100, 150, 250 and 500 μ M MTX

(1 μ M MTX stock solution)

- 1.) 346 TNF α 2.3 / 112995 p04
- 2.) 346 TNF α 4.1 / 112995 p04
- 3.) 346 TNF α 4.3 / 112995 p04
- 4.) 346 TNF α 4.4 / 112995 p04
- 5.) 346 TNF α 4.5 / 112995 p04
- 6.) 346 TNF α 4.6 / 112995 p04
- 7.) 346 TNF α 4.7 / 112995 p04
- 8.) 346 TNF α 4.10 / 112995 p04
- 9.) 346 TNF α 5.3 / 112995 p04
- 10.) 346 TNF α 5.4 / 112995 p04

2.16.96 CHO anti-DG44 passaging of cells

CHO anti-DG44 / 010996 p15 split ratio 1:10
in MEM, 5% FBS, 1% PS

Establishing of stable 346 VIRFS clone

amplification up to 100 μ M MTX
continued from p. 64

- remove medium in T75 flask and replace with
fresh MEM, 5% FBS, 1% PS and 80 μ M MTX

Comparative study 396 ST5, CHO ST10.1

2.16.96

Take 3x1ml aliquots of each clone and passage cells
in MEM, 5% O.FBS, 1% PS, 80 μ M MTX

- 1.) 396 ST5 / 011696 p09
- 2.) CHO ST10.1 / 011696 p09

2.20.96

Comparative study 396 ST5, CHO ST10.1

Take 3x1ml aliquots of each clone and passage cells
in MEM, 5% O.FBS, 1% PS, 80 μ M MTX

- 1.) 396 ST5 / 011696 p10
- 2.) CHO ST10.1 / 011696 p10

Establishing of stable 396 VEGF clone

- amplification up to 80 μ M MTX
- passage cells and seed in 2 T25 flasks
for freezing and analysis of cells
in MEM, 5% O.FBS, 1% PS, 80 μ M MTX

Establishing of stable 396 TNF clones (continued p. 70)

- expanding of backup clones for freezing
passage cells from T25 flasks into T75 flasks
in MEM, 5% O.FBS, 1% PS, 20 μ M MTX

- | | |
|------------------------------|--------------------------------|
| 1.) 396 TNF 4.2 / 112995 p04 | 7.) 396 TNF 6.6 / 112995 p03 |
| 2.) 396 TNF 4.8 / 112995 p04 | 8.) 396 TNF 6.7 / 112995 p03 |
| 3.) 396 TNF 4.9 / 112995 p04 | 9.) 396 TNF 6.8 / 112995 p03 |
| 4.) 396 TNF 5.1 / 112995 p04 | 10.) 396 TNF 6.9 / 112995 p03 |
| 5.) 396 TNF 5.2 / 112995 p04 | 11.) 396 TNF 6.10 / 112995 p03 |
| 6.) 396 TNF 6.3 / 112995 p03 | |

2.20.96

for two of the 346 TNF γ clones the medium was replaced by MEM α , 5% DFB, 1% PS, 20 nM MTX

- 1.) 346 TNF γ 6.1 / 112995 p 02
- 2.) 346 TNF γ 6.4 / 112995 p 02

Passaging of CHO^{dhfr} DG44 cells

- 1.) passage cells in T 225 flasks CHO^{dhfr} DG44 / 010996 p16
split ratio 1:8
in MEM α , 5% DFB, 1% PS (2.20.96)

- 2.) resuspend remaining cells in a final volume of ~~20~~ 10 ml

resuspend 3 ml of this cell suspension in 13.5 ml final volume

add 1 ml of this dilution to each of the wells of 2-6 well dishes for transfection

Establishing of stable CHO TNF γ cell lines

Amplification of clones: continued p. 71

cells growing at 0 - 1000 nM MTX,
pool cells in several wells and seed at
0, 100, 200, 500, 800 and 1000 nM MTX

- 1.) CHO TNF γ 1.2 / 112995 p09
- 2.) CHO TNF γ 1.3 / 112995 p09
- 3.) CHO TNF γ 1.7 / 112995 p09
- 4.) CHO TNF γ 1.9 / 112995 p09
- 5.) CHO TNF γ 2.7 / 112995 p09

cells growing at 0-500 mM NaCl, pooled and
seeded at 0, 100, 200, 500, 800 and 1000 mM NaCl

- 1.) CHO TNG⁺ 1.6 / 112995 p08
- 2.) CHO TNG⁺ 2.3 / 112995 p08
- 3.) CHO TNG⁺ 2.9 / 112995 p08
- 4.) CHO TNG⁺ 6.8 / 112995 p06
- 5.) CHO TNG⁺ 6.9 / 112995 p07
- 6.) CHO TNG⁺ 6.7 / 112995 p07

Establishing of new stable CHO cell lines

2.21.96

Transfection of CHO^{dhfr}-DR44 cells with
expression vectors:

- 1.) CESP / HITFHS78 / Project Code HG11600 / pCI (Dan Sappel)
- 2.) GDF / HITER36 / Project Code HG11000 / pCI (Dan Sappel)
- 3.) VEGF2 / HOSBD47 / Project Code HG00400 / pCI (Jing-Sen He)
- 4.) TGF- β 1 / HE9CC44 / Project Code HG07000 / pCI (Jing-Sen He)
- 5.) Control pN18 only
- 6.) control no DNA added to cells

CHO^{dhfr}-DR44 / DR44 / 010996 p16 cells were seeded
for transfections in 96 wells at 6 wells per day before
cells were 20-80% confluent

- remove TCM, wash cells 1x with PBS, remove PBS
- add 1 ml of Opti-MEM + 1% PS to each well
- prepare for each transfection reaction in 96 wells at 6 wells per day

A: 90 μ l Opti-MEM
10 μ l Lipofectin

B: 50 μ l Opti-MEM, 5 μ g Expression vector, 0.5 μ g pN18

- Mix A and B and incubate at RT for 45'
- Add Transfection mix to the cells and incubate for 55' at 37°C, 5% CO₂.
- Add 1ml of Opti MEM + 10% FBS + 1% PS

2.22.96 Establishing of CHO-TNF α clones and 346-TNF α clones

Initial screening for high expressing clones by Western analysis

- Sample preparations: all TNF α clones were incubated for 2 days in CHO-S-SEM-II + 1% PS before the medium was harvested and frozen.
 see p. 48 and p. 56 for CHO-TNF α clones
 see p. 70 for 346-TNF α clones
- 11 CHO-TNF α clones, that in parallel are amplified and 10 x 346-TNF α clones were tested for TNF α expression in Western blots
- Samples of each clone were loaded on a 16% NOVEX Gel
 - a) 30 μ l medium straight
 - b) 500 μ l medium TCA precipitated

TCA - Precipitation:

- 500 μ l supernatant
- + 50 μ l 0.15% DOC
- + 70 μ l 100% TCA
- vortex 5' on ice
- 5' 14000 RPM
- remove sup. add 100 μ l chloroform
- 2' on ice
- 5' 14000 RPM
- resuspend pellet in 30 μ l 0.1N HCl + 0.1% SDS
- add 10 μ l 4X SDS loading buffer

positive controls: 1.) samples that were not precipitated (straight)
 50 μ g E. coli expressed a α purified (when ok)
 TNF β was added to 30 μ l of
 CHO-S-SFM-II + 10 μ l sample buffer

2.) precipitated samples: (precip.)

50 μ g TNF β was added to 500 μ l
 CHO-S-SFM-II and the TCA precip.
 was done as described above

CHO-TNF β clones

all gels 16% NOVEX gels

Gel 1: 1) M

2) CHO-TNF β 1.2	30 μ l straight
3) CHO-TNF β 1.3	-11-
4) CHO-TNF β 1.7	-11-
5) CHO-TNF β 1.9	-11-
6) CHO-TNF β 2.7	-11-
7) CHO-TNF β 1.6	-11-
8) CHO-ST10.1	-11-
9) 50 μ g TNF β	straight
10) M	

Gel 2: 1) M

2) CHO-TNF β 6.4	30 μ l straight
3) CHO-TNF β 6.7	-11-
4) CHO-TNF β 6.8	-11-
5) CHO-TNF β 2.3	-11-
6) CHO-TNF β 2.9	-11-
7) CHO-TNFβ 50 μ g TNF β	straight
8) CHO-ST10.1	30 μ l straight
9) M	
10) M	

I. CHO TNEF clones -Gel 3:

- 1.) M
- 2.) CHO TNEF 1.2 ^{500µl precipitated} ~~200µl straight~~
- 3.) CHO TNEF 1.3 -11-
- 4.) CHO TNEF 1.7 -11-
- 5.) CHO TNEF 1.9 -11-
- 6.) CHO TNEF 2.7 -11-
- 7.) CHO TNEF 1.6 -11-
- 8.) CHO ST 10.1 -11-
- 9.) 50mg TNEF 30µl straight
- 10.) M

Gel 4:

- 1.) M
- 2.) CHO TNEF 6.4 500µl precip.
- 3.) CHO TNEF 6.7 500µl precip.
- 4.) CHO TNEF 6.8 500µl precip.
- 5.) CHO TNEF 2.3 500µl precip.
- 6.) CHO TNEF 2.9 500µl precip.
- 7.) CHO ST 10.1 500µl precip.
- 8.) 50mg TNEF straight
- 9.) 50mg TNEF precipitated
- 10.) M

II 346 TNEF samples:Gel 1:

- 1.) M
- 2.) 346 TNEF 2.3 30µl straight
- 3.) 346 TNEF 4.1 30µl straight
- 4.) 346 TNEF 4.3 30µl straight
- 5.) 346 TNEF 4.4 30µl straight
- 6.) 346 TNEF 4.5 30µl straight
- 7.) CHO ST 10.1 30µl straight
- 8.) 50mg TNEF straight
- 9.) M
- 10.) M

Gel 2:

- 1.) M
- 2.) 346 TNEF 4.6 30µl straight
- 3.) 346 TNEF 4.7 30µl straight
- 4.) 346 TNEF 4.10 30µl straight
- 5.) 346 TNEF 5.3 30µl straight
- 6.) 346 TNEF 5.4 30µl straight
- 7.) ~~CHO ST 10.1~~ CHO ST 10.1 30µl sh.
- 8.) ~~50mg TNEF~~ 50mg TNEF straight
- 9.) M
- 10.) M

2.22.96 JMB

II 346 TWEY samples:

Gel 3:

- 1.) μ
- 2.) 346 TWEY 2.3 500 μ l precipit.
- 3.) 346 TWEY 4.1 500 μ l precipit.
- 4.) 346 TWEY 4.3 500 μ l precipit.
- 5.) 346 TWEY 4.4 500 μ l precipit.
- 6.) 346 TWEY 4.5 500 μ l precipit.
- 7.) CHO ST 10.1 500 μ l precipit.
- 8.) 50mg TWEY straight
- 9.) 50mg TWEY precipit.
- 10.) μ

Gel 4:

- 1.) μ
- 2.) 346 TWEY 4.6 500 μ l precip.
- 3.) 346 TWEY 4.7 500 μ l precip.
- 4.) 346 TWEY 4.10 500 μ l precip.
- 5.) 346 TWEY 5.3 500 μ l precip.
- 6.) 346 TWEY 5.4 500 μ l precip.
- 7.) CHO ST 10.1 500 μ l precip.
- 8.) 50mg TWEY straight
- 9.) 50mg TWEY precip.
- 10.) μ

Western blot: Nitrocellulose Membrane

- preset memb. in blotting buffer
- electrotransfer at 30V 1h
- 1h blocking at 4°C, 4% milk, rinsed 3x memb.
- 2.5 incubation with 1st AP

TTX (see p. 62) 1:5000

- rinse 2x with 1x PBS and wash 3x with 1x PBS, 10'
- 1h incubation with 2nd AP 1:2000 (dilute 1st AP)

- Result Western: W needs dose optimization, transfer time

- 1.) - Background reduction, use TWEEN-PPS
- 2.) - 1st AP incubation over night
- 3.) - Antibody specificity: AP was derived from E. coli expressed material, precipit.

CHO TNE-2 clones (112995 p.k.) Western first screening
(samples: 200 µl TCH)

I2
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
 2

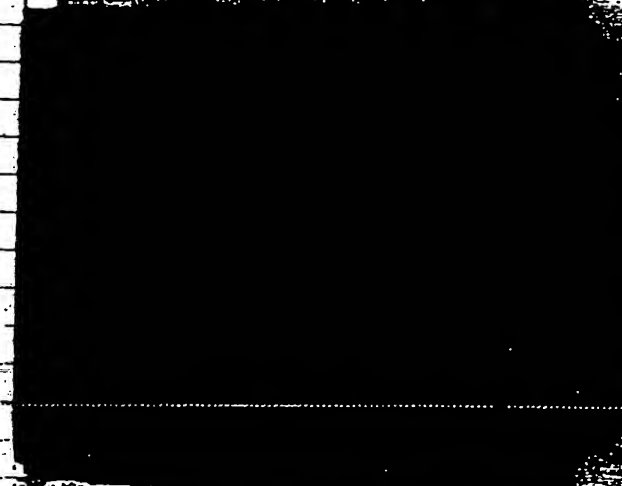
98
64
50
36
30
16

98
64
50
36
30
16



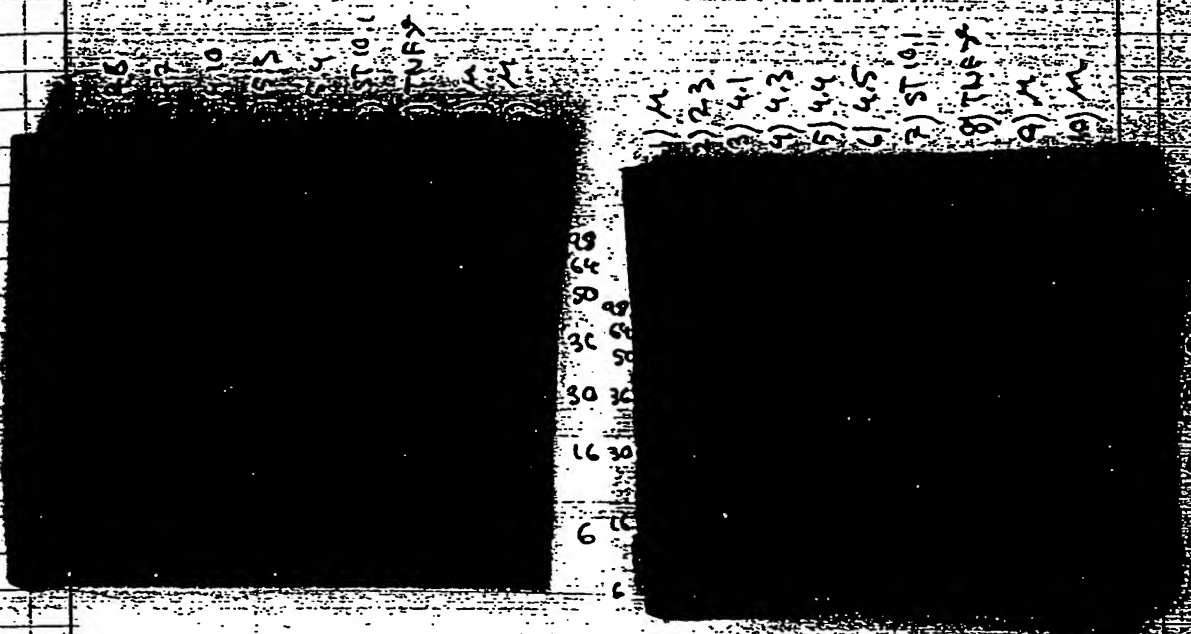
CHO TNE-2 clones (112995 p.k.) Western first screening
(samples: 500 µl TCH TCH-precip.)

I3
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

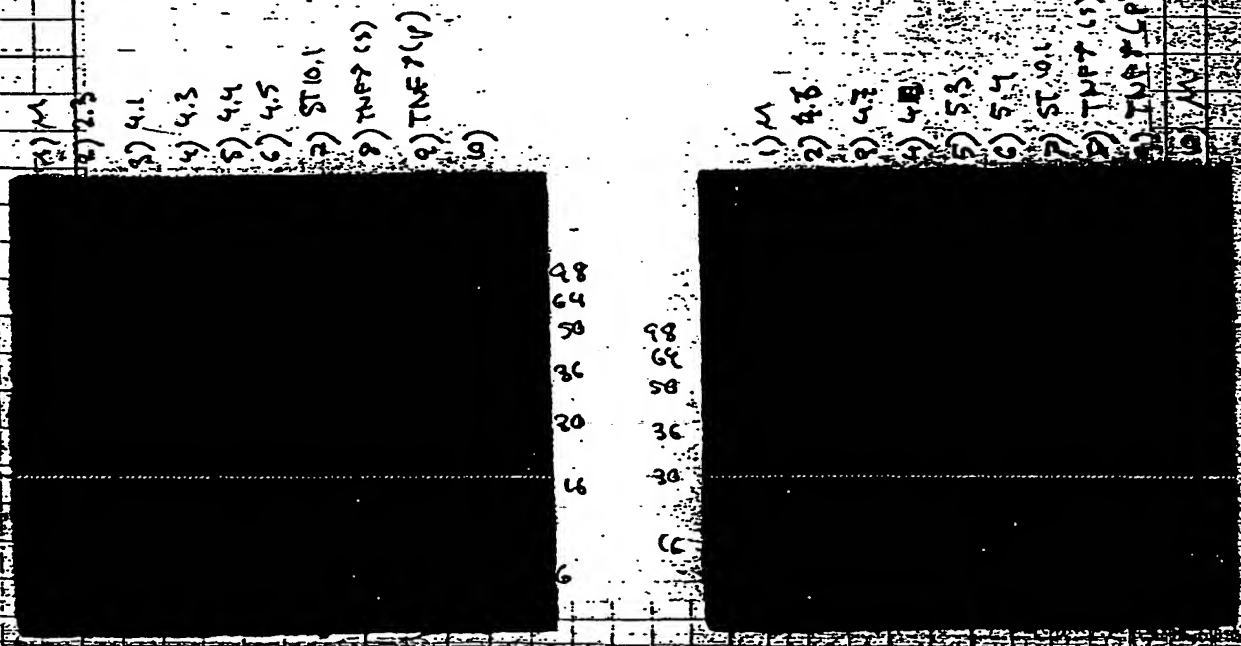


98
64
50
36
30
16

346 TNF8 clone / 112995 px / Western first screening
 (samples 30 µl TCM strip 5)



CHO TNF8 clone / 112995 px / Western first screening



2.23.96

Establishing of stable CHO clones:

CESP, GDF, VEGF2, FGF II

continued from P. 75

Selection of recombinant CHO-clones for amplification

- Cells that were transduced in Gwell dishes (see p. 75) were seeded in 3 96-well plates each clone in MEM, 15% FBS, 1% PS, 1000 U/ml, 1 mg/ml G418.

96-well plating:

1.) CESP-1 / 022196 p01

2.) CESP-2 / 022196 p01

3.) CESP-3 / 022196 p01

4.) GDF-1 / 022196 p01

5.) GDF-2 / 022196 p01

6.) GDF-3 / 022196 p01

7.) VEGF2-1 / 022196 p01

8.) VEGF2-2 / 022196 p01

9.) VEGF2-3 / 022196 p01

10.) FGF-II-1 / 022196 p01

11.) FGF-II-2 / 022196 p01

12.) FGF-II-3 / 022196 p01

13.) negative control N18-1

14.) -II- N18-2

15.) -II- N18-3

16.) -II- -1 (no DNA)

17.) -II- -2 (no DNA)

18.) -II- -3 (no DNA)

CHO atk - 10644/

2.23.96

CHO atk - 10644/ 010996 p14 split 1:8

passage into MEMU, SY-DEBS, 1% PS

SUPERVISOR - Junia Zhang
DATE - 2/23/96

Compensated Study 346 STS, CHO ST10.1

2.23.96

Use 3x 1ml aliquots of each clone and passage cells
split 1:3 in MEMU, SY-DEBS, 1% PS, 80% NIX

- 1.) 346 STS 1011696 p11 (freeze cells 2.23.96)
- 2.) CHO ST10.1/011696 p11 (" ")

Establishing of stable 346 VIRFS clone

- amplification up to 80% NIX
- passage cells and seed in 2x T25 flasks
- for freezing and analyzing

Establishing of stable CHO clones 346 TNE2*

2.24.96

(continued from p. 73)

freezing of backup clones

- 1.) 346 TNE2* 6.8 p.03
- 2.) 346 TNE2* 6.9 p.03
- 3.) 346 TNE2* 6.10 p.03
- 4.) 346 TNE2* 6.9 p.04
- 5.) 346 TNE2* 6.8 p.04
- 6.) 346 TNE2* 4.2 / 112995 p.04
- 7.) 346 TNE2* 4.7 / 112995 p.03
- 8.) 346 TNE2* 6.3 / 112995 p.03
- 9.) 346 TNE2* 6.6 / 112995 p.03
- 10.) 346 TNE2* 5.1 / 112995 p.04
- 11.) 346 TNE2* 5.2 / 112995 p.04

2.24.96 Establishing stable CHO TNE⁺ cell linesAmplification continued from p 74, 75

cells were growing at 100 - 1000 nM MIX
 pools confluent wells at 80 nM and 1000 nM and
 seed in 0; 0.5; 1; 15; 8 and 5 μ M MIX.

- 1.) CHO TNE⁺ 2.3 / 112995 p09
- 2.) CHO TNE⁺ 2.7 / 112995 p10
- 3.) CHO TNE⁺ 4.3 / 112995 p10
- 4.) CHO TNE⁺ 6.4 / 112995 p8
- 5.) CHO TNE⁺ 6.7 / 112995 p08

2.26.96 Comparative Study ST5, ST 10.1

take 3 x 1ml aliquots each clone and passage
 cells 1:4 in MEM⁺, 5% FBS, 1% PS, 80 μ M MIX

- 1.) 346 ST5 / 01696 p 12
- 2.) CHO ST10.1 / 01696 p 12

CHO d₁₀ / 0644CHO d₁₀ / 0644 / 010996 p 15 Spet 1:10MEM⁺, 5% FBS, 1% PS,5mM MIX Stock solution:

- dissolve 500mg MIX in 44ml MEM⁺ - Medium
- filter/sterilize through a 0.2 μ m filter
- aliquot in 14 x 1ml aliquots and 6 x 5ml aliquots

Establishing of stable 3T3 TNEF clones

2.27.96

Amplification continued from p 72

- cells grow at low density, many dead cells in suspension
- remove supernatant and wash each well with 2ml PBS
- add 2ml of MEM, 5% FBS, 1% PS to each well to recover cells at all different wells

Establishing stable CHO TNEF clones

2.29.96

continued p. 84 amplification

cells were growing at concentrations between 0 and 5 μ M MTA

- 1.) pool cells at all concentrations in 7ml final volume
- 0) - seed 1 ml in each well of a 6 well dish at 0; 0.5; 1; 1.5; 3 and 5 μ M MTA
- 1) - seed 1 ml into a T25 flask in MEM⁺ and expand for testing (base line etc.)

- 1.) CHO TNEF 2.3 / 112995 p0 *
- 2.) CHO TNEF 2.7 / 112995 p0
- 3.) CHO TNEF 4.3 / 112995 p0
- 4.) CHO TNEF 6.4 / 112995 p09
- 5.) CHO TNEF 6.7 / 112995 p09

2.27.96 MB

2.29.96 Establishing stable CHO TNF α clones

continued from p 74 Amplification

- 1.) - cells were growing at 0-1000 nM MTX
- dead cells in suspension wells 40-50% confluent
- wash with PBS and passage in 6 well dishes at 0-1000 nM MTX

1) CHO TNF α 1.6 / 112995 p092) CHO TNF α 6.8 / 112995 p073) CHO TNF α 1.2 / 112995 p04) CHO TNF α 1.9 / 112995 p90

- 2.) wash with PBS and replace TCM (same as 0 MTX)

1.) CHO TNF α 1.7 / 112995 p092.) CHO TNF α 2.9 / 112995 p083.1.96 Establishing stable 346 TNF α cell lines

continued from p 85

Amplification cells that grew at MTX concentr. from 50 to 500 nM before were growing in MEM α medium without MTX to recover and expand the cells (see p. 85)

The cells were passed and selected in 0, 100, 200, 500, 800 and 1000 nM MTX

1.) 346 TNF α 2.3 / 112995 p052.) 346 TNF α 9.1 / 112995 p053.) 346 TNF α 4.3 / 112995 p054.) 346 TNF α 4.4 / 112995 p055.) 346 TNF α 4.5 / 112995 p056.) 346 TNF α 4.6 / 112995 p057.) 346 TNF α 4.7 / 112995 p058.) 346 TNF α 4.10 / 112995 p05

- 2.) 346 TNA⁺ 5.3 / 112985 pos
 10.) 346 TNA⁺ 5.4 / 112985 pos

Comparative Study ST5, ST 10.1

take 3 x 1ml aliquots each clone and passage cells
 1:3 in MEM⁺, 5% ABSS, 1% PS, 80 μ M MTX

- 1.) 346 ST5 / 011696 p13
 2.) 346 ST10.1 / 011696 p13

Duplication of VIGF 5

from p13 - passage cells in MEM⁺, 5% ABSS, 1% PS
 80 μ M MTX
 (for freezing and labeling)

Comparative Study of serial VIGF and TNA⁺ clones (Labeling with 35S-Cys + 35Meth)

Trans clones:

freezing date:

- | | | |
|-----------------------------|---------|--------------------|
| 1.) ST5 (100 μ M MTX) | 10.5.95 | ST5 / 030196 p01 |
| 2.) VIGF5 (5 μ M MTX) | 12.2.95 | VIGF5 / 030196 p01 |
| 3.) VIGF3 (100 μ M MTX) | 12.2.95 | VIGF3 / 030196 p01 |

Seed cells in T25 at 80 μ M resp. 1 μ M MTX

CHO cells - DG44 / 010992 p16 Split 1:10
 MEM⁺, 5% ABSS, 1% PS

3.1.96 MR

VGIF3 received from clinic seeded in T75 for labeling:

VGIF3 p.15

Labeling of ST, VGIF and TNF α cells

3.4.96

Prepare cells for labeling experiment:

- cells were seeded in T75 flasks before (see previous pages)
- for labeling experiment seed cells into the wells of a 6 well dish in MEM α , 5% FBS, 1% PS

	T75 flasks	into 6 well
1.) 346 STS 1030196 p.02	70%	1:7
2.) VGIF3 (5 μ M) 1030196 p.02	100%	1:10
3.) VGIF3 (100 μ M) 1	80%	1:8
4.) VGIF3 1030196 p.02	80%	1:8
5.) VGIF3 1 p.16	100%	1:10
6.) CHOTNF α 2.7 / 112995 p.12	20%	1:2
7.) CHOTNF α 4.3 / 112995 p.12	30%	1:3
8.) CHOTNF α 6.9 / 112995 p.10	50%	1:5
9.) CHOTNF α 6.7 / 112995 p.10	70%	1:7

Comparative study STS, ST10.1

- use 3x 1ml aliquots of each clone and passage cells 1:4 in MEM α , 5% FBS, 1% PS, 80 μ M MEM

346 STS 1041696 p.14

CHOTNF α 1041696 p.14

Establishing stable 346 TNF α cell lines

3.5.96

continued from p. 86

cells growing from 0 - 1000 μ M MTX. cells
are confluent at 800 μ M and 1 μ M
pool cells at 800 μ M + 1 μ M and seed into 6
wells of a 6 well at 0/0.5/1/1.5/3
and 5 μ M.

- 1.) 346 TNF α / 112995 p06 / 2.3
- 2.) 346 TNF α / 112995 p06 / 4.1
- 3.) 346 TNF α / 112995 p06 / 2.3
- 4.) 346 TNF α / 112995 p06 / 4.4
- 5.) 346 TNF α / 112995 p06 / 4.5
- 6.) 346 TNF α / 112995 p06 / 4.6
- 7.) 346 TNF α / 112995 p06 / 4.7
- 8.) 346 TNF α / 112995 p06 / 4.10
- 9.) 346 TNF α / 112995 p06 / 5.3
- 10.) 346 TNF α / 112995 p06 / 5.4

Establishing stable CHO TNF α cell lines

3.5.96

continued from p. 86

cells growing up to 1 μ M MTX

pool cells at different concentrations and seed
in 0/0.5/1/1.5/3 and 5 μ M MTX

- 1.) CHO TNF α 1.7 / 112995 p10
- 2.) CHO TNF α 6.8 / 112995 p08
- 3.) CHO TNF α 2.9 / 112995 p09

3.5.96 MB

3.5.96

Labeling of ST, VIGF and TNF γ cell lines

- 1.) 346 ST5
- 2.) VIGF3 (5 μ M)
- 3.) VIGF3 (10 μ M MIX)
- 4.) VIGF3 / 030196 p02
- 5.) VIGF3 / p16
- 6.) CHO TNF γ 2.7 / 112995 p12
- 7.) CHO TNF γ 4.3 / 112995 p12
- 8.) CHO TNF γ 6.4 / 112995 p10
- 9.) CHO TNF γ 6.7 / 112995 p10
- 10.)

cells are confluent in 6 well dishes

add to each well:add: 35 S - Glysine and 35 S-Methionine5 μ l of each at 10 cells/well (10 μ l / well)

3.6.96

Establishing of recombinant CHO TNF γ clonescontinued from P. 85 Amplificationcells are growing at 0 - 5 μ M MIX

Pool cells in 6 wells at

0 / 0.5 / 1 / 1.5 / 3 and 5 μ M MIX

- 1.) CHO TNF γ 2.3 / 112995 p11 (pool all wells)
- 2.) CHO TNF γ 2.7 / 112995 p12 (pool 1 μ M - 5 μ M MIX)
- 3.) CHO TNF γ 4.3 / 112995 p12 -11-
- 4.) CHO TNF γ 6.4 / 112995 p10 -11-
- 5.) CHO TNF γ 6.7 / 112995 p10 (pool 3 μ M + 5 μ M)

Testing:

for testing in the antituberc assay cells of the following clones were seeded into 8 T25 flasks in MEM⁻, OMTX, 5% aFBS, 1% PS:

- 1.) CHO TNEF 2.2 / 112995 p 12 (0.5 μ M MTX)
- 2.) CHO TNEF 4.3 / 112995 p 12 (0.5 μ M MTX)
- 3.) CHO TNEF 6.4 / 112995 p 10 (0.5 μ M MTX)
- 4.) CHO TNEF 6.2 / 112995 p 10

Establishing of stable CHO TNEF clones:

3.6.95

continued from p 86:

cells were growing at 0-1000 nM MTX before -
~~(the medium was not changed for 2 weeks)~~

- 1) cells that were growing at 300 nM 500 nM and 1 μ M were pooled and seeded in 0 / 500 / 1000 / 1500 / 3000 and 5000 nM MTX

- 1.) CHO TNEF 1.6 / 112996 p 10
- 2.) CHO TNEF 1.9 / 112996 p 4
- 3.) CHO TNEF 1.2 / 112996 p 4

- 2) Prepare cells for testing:

cells growing at 200 nM MTX were seeded into a T25 flask in MEM⁻, OMTX, 5% aFBS, 1% PS

for testing in the antituberc assay -

- 1.) CHO TNEF 1.6 / 112996 p 10
- 2.) CHO TNEF 1.9 / 112996 p 4
- 3.) CHO TNEF 1.2 / 112996 p 11

S.A. 96MP

3.11.96

Establishing of stable CHO TNF γ cell linescontinued from p. ~~88~~, ~~89~~, 89, 90, 91Amplification

- 1) CHO TNF γ 1.2 / 112995 p 12
- 2) CHO TNF γ 1.6 / 112995 p 11
- 3) CHO TNF γ 1.7 / 112995 p 11
- 4) CHO TNF γ 1.9 / 112995 p 12
- 5) CHO TNF γ 2.3 / 112995 p 12
- 6) CHO TNF γ 2.7 / 112995 p 13
- 7) CHO TNF γ 2.9 / 112995 p 10
- * 8) CHO TNF γ 4.3 / 112995 p 13
- * 9) CHO TNF γ 6.4 / 112995 p 11
- * 10) CHO TNF γ 6.7 / 112995 p 11
- * 11) CHO TNF γ 6.8 / 112995 p 09

cells were growing in 6 wells 0-5 μ M MTX
 pooled cells at 2998 clone and seed in
 0.05, 1.5, 3 and 5 μ M MTX

Establishing stable 346 TNF α cell lines

3.12.96

continued from p. 89

1.) Harvest supernatants for artificial assay
(Don Redmanek)

2.) pool cells and seed at 0-5 μ M MTX
(2nd time free concn.)

1.) Harvest for testing: (see p. 89)

- | | | |
|-----------------------------|-----|--|
| 1.) 346 TNF α 2.3 / | p06 | (cells were growing at
0-1 μ M MTX before
seeded in selective MTX) |
| 2.) 346 TNF α 4.1 / | p06 | |
| 3.) 346 TNF α 4.3 / | p06 | |
| 4.) 346 TNF α 4.4 / | p06 | |
| 5.) 346 TNF α 4.5 / | p06 | |
| 6.) 346 TNF α 4.6 / | p06 | |
| 7.) 346 TNF α 4.7 / | p06 | |
| 8.) 346 TNF α 4.10 / | p06 | |
| 9.) 346 TNF α 5.3 / | p06 | |
| 10.) 346 TNF α 5.4 / | p06 | |

cells each clone growing in 6 wells at different
MTX concentrations. (0-5 μ M)

harvest sup of each clone growing at 0 MTX
and submit for artificial assay.

2.) seed cells in new 6 well dishes for amplification.

- | | |
|----------------------------|------------|
| 1.) 346 TNF α 2.3 / | 112995 p02 |
| 2.) 346 TNF α 4.1 / | 112995 p02 |
| 3.) 346 TNF α 4.3 / | 112995 p02 |
| 4.) 346 TNF α 4.4 / | 112995 p02 |
| 5.) 346 TNF α 4.5 / | 112995 p02 |

- 6.) 396 TNEF 8.6 / 112995 p07
- 7.) 396 TNEF 8.7 / 112995 p07
- 8.) 396 TNEF 8.10 / 112995 p07
- 9.) 396 TNEF 8.3 / 112995 p07
- 10.) 396 TNEF 8.4 / 112995 p07

cells growing at different concentrations were pooled and seeded at 0, 0.5, 1, 1.5, 3 and 5 μ M MTX

3.12.96 CHO TNEF samples submitted for anti-CR1 assay (Dan Beckman's)

continued from P. 91

clones were growing in T25 flasks in MEM + 0 MTX, 5% FBS, 1% PS

- | | | |
|-----------------------------|-----|------------------------------|
| 1.) CHO TNEF 1.2 / 112995 | p11 | seeded from: 0.2 μ M MTX |
| 2.) CHO TNEF 1.6 / 112995 | p10 | 0.2 μ M MTX |
| 3.) CHO TNEF 1.9 / 112995 | p11 | 0.2 μ M MTX |
| 4.) CHO TNEF 2.7 / 112995 | p12 | 0.5 μ M MTX |
| * 5.) CHO TNEF 4.3 / 112995 | p12 | 0.5 μ M MTX |
| 6.) CHO TNEF 6.4 / 112995 | p10 | 0.5 μ M MTX |
| 7.) CHO TNEF 6.7 / 112995 | p10 | 0.5 μ M MTX |

* clone CHO TNEF 4.3 was mislabeled on tube that was submitted to Dana, its label: CHO TNEF 4.3

3.12.96 up

Comparative Study ST5, ST10.1

313.96

from p 94:

harvest 3 x 1 ml aliquots of each clone and freeze at -80°C

Passage cells 1:3 in R T75 test tubes alone at MEM, 5% DFB, 1% PS, 80 µM MTA

- 1.) 346 ST5 / 011696 p15
- 2.) CHO ST10.1 / 011696 p15

Amplification of VGF S up to 100 µM MTA:

from p. 87:

- passage cells 1:3 in T75 test tubes in MEM, 5% DFB, 1% PS, 80 µM MTA

VGF S / 031396 p00

Breeding of ST5, ST10.1 and VGF S clones from above:

313.96

freeze 3 x 1 ml aliquots of 10⁶ cells at

- 1.) 346 ST5 / 011696 p15 at 80 µM MTA
- 2.) CHO ST10.1 / 011696 p15 at 80 µM MTA
- 3.) VGF S / 031396 p00 at 80 µM MTA

(protocol see p50)

313.96

3.13.96 Establishing of FGF11 clones:

continued from p 82

Project Code HG 07000
HE9CC44

passaging of 48 clones from Hybridoma plates into 24 wells into MEMT, 5% DMSO, 1% PS, 10mM MTX

see p. 92

FGF11 - 11022196 p02 up to FGF11-48/022196 p02

3.14.96 Establishing of stable CHO TNEF8 cell lines

Continued from p. 96

Amplification:

- Cells confluent in 5 μ M MTX:
- pool cells in 3 μ M MTX and 5 μ M MTX
- seed 50% of the pooled cells in 6 wells at 96 well dishes in 0, 2.5, 5, 7.5, 12.5 and 25 μ M MTX
- first seeded at these concentrations

1.) CHO TNEF8 1.3 / 112996 p. 14

2.) CHO TNEF8 16.4 / 112996 p. 12

3.) CHO TNEF8 6.7 / 112996 p. 12

4.) CHO TNEF8 6.8 / 112996 p. 18

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